Additional lectin receptors in galactans from the albumin gland of the *Achatina fulica* snail

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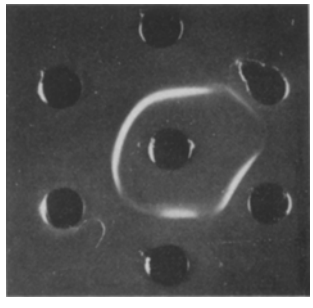
Summary. The lectin receptor-site specificity of a purified galactan from snail *(Achatina fulica)* albumin glands has been studied by precipitin reactions in agar-gel double diffusion experiments with different lectins. Most lectins were found to be specific for terminal β-D-galactose structures. Some findings suggest, that the structure DGalβ1→3DGal may be one of the receptor sites on the polysaccharide.

In previous communications we have demonstrated the presence of different receptors for heterophile lectins from plant, invertebrate and microbial origin on the proteogalactans from snail *(Achatina fulica)* albumin glands[2-4]. Most of these lectins are galactose specific (for an extensive review see Uhlenbruck[5]). This paper reports additional investigations on the lectin receptor sites of a purified galactan from this source.

The results of the agar-gel double diffusion experiments with the homogeneous galactan (polyacrylamide gel electrophoresis) isolated from the total proteogalactans by affinity chromatography on agarose-peanut lectin beads[6], when tested against different galactose-binding lectins, are presented in the table.

Whereas no precipitin lines were formed by some Tridacnin and Axinella lectins, both with anti-DGalβ1→6DGal specificity, the myeloma protein J 539 did react. Visible but weak precipitin lines of non-identity were formed by *Abrus precatorius* (not by *Phaseolus vulgaris*) and the cholera lectin (choleratoxin, B-fragment), which reacts with the terminal carbohydrate of the ganglioside GM_1 (fig.). These observations indicate additional lectin receptors on the sialic acid free galactans. Strong precipitin lines were formed by the lectins from *Ricinus communis* (RCA_{60}, RCA_{120}), *Tridacna squamosa, Tridacna derasa, Tridacna crocea.* These lectins also possess β-galactosyl specificity. The precipitin lines, however, exhibited close identity indicating similar combining sites for these lectins. Similar results were obtained with the peanut lectin (PNA) and the lectin from *Bauhinia purpurea alba* seeds (fig.). The latter finding means another interesting new lectin receptor site on these galactans.

The disaccharide DGalβ1→3DGalNAc has been considered as the dominant receptor structure for the peanut lectin[7]. Recently, after elegant immunochemical studies on the combining site of the *Bauhinia purpurea* lectin, the structure DGalβ1→3DGalNAcβ1→3DGal has been proposed[8]. Neither the total proteogalactans nor the galactan from the affinity experiment contained, however, detect-



Agar-gel diffusion pattern of *Achatina fulica* galactan (center) with galactose specific lectins starting at 12 o' clock and moving clockwise. 1. *Bauhinia purpurea* lectin; 2. Myeloma protein J 539; 3. *Abrus precatorius;* 4. *Glycine max;* 5. Cholera lectin; 6. *Arachis hypogaea;* 1% solution in saline of the lectins and of the galactan.

Precipitin reactions in agar of galactans from the albumin glands of *Achatina fulica* with different anti-galactose lectins

	Lectin	Origin	Specificity	Reaction with galactan
1	*Arachis hypogaea*	P	DGalß1->3DGalNAc	+
2	*Bauhinia purpurea*	P	DGalß1->3DGalNAcß1->3DGal	+
3	Choleralectin	B	DGalß1->3DGalNAcß1->4DGal	+
4	Tridacnins (x)			
4a	*T. maxima*, *T. gigas*	I	DGalß1->6	ø
4b	*T. crocea*, *T. derasa*, *T. squamosa*	I	DGalß1->4?	+
5	*Ricinus communis* I	P	DGalß1->?	+
6	*Ricinus communis* II	P	DGalß1->?	+
7	*Axinella polypoides* (I and II)	S	DGalß1->6DGal	ø
8	*Agaricus bisporus*	P	DGalß1->?	ø
9	*Phaseolus vulgaris*	P	DGalß1->4	ø
10	*Abrus precatorius*	P	DGalß1->4	+
11	*Viscum album* (x)	P	DGalß1->?	+
12	Myeloma protein J 539	V	DGalß1->6DGal	+
13	*Pseudomonas aeruginosa*	B	DGalß1->?	ø
14	*Wistaria floribunda*	P	DGalNAcα1->6DGal	+
15	*Glycine max*	P	DGalNAcα1->3DGalß1->3DGal	+
16	*Geodia cydonium* (x)	S	DGalß1->4	ø
17	*Cerianthus membranaceus* (x)	I	DGalß1->?	+
18	C-reactive protein	V	DGalß1->?	ø

+, Precipitin line; ø, no visible reaction; P, plant; I, invertebrate; S, sponge; V, vertebrate; B, bacteria; (x), own preparation.

able amounts of hexosamine[6]. The precipitin lines do represent the lectin-glycoconjugate complexes. As unspecific reactions may occur in this Ouchterlony agar-gel technique, as discussed earlier by us[9], these were completely excluded in this investigation by control experiments. Moreover, lectins purified by us, or from commercial sources (Medac) were employed in this investigation. Accordingly, the interaction between the lectin and the galactan can be supposed to be specific.

It has been claimed that the Agaricus lectin has a very similar specificity to that of the peanut lectin, namely DGalβ1→3DGalNAc[10]. Experiments from this laboratory could, however, not confirm this observation[11,12]. Again, in the present experiments, the Agaricus lectin unlike the peanut one, did not react with the snail galactan. Whereas, therefore, the exact specificity of the Agaricus lectin has still to be defined, the reactions of the Arachis lectin with the galactan can only be interpreted as being due to a hexosamine-free DGalβ1→3DGal terminal disaccharide, because β1→4 linkages, which may also react with peanut[12], do not usually occur in snail galactans[13], which mainly have β1→6 or β1→3 galactosidic linkages[13,14].

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Specific immunosuppression of IgE response to hapten DNP by DNP linked to monoclonal IgG_1 in rats[1]

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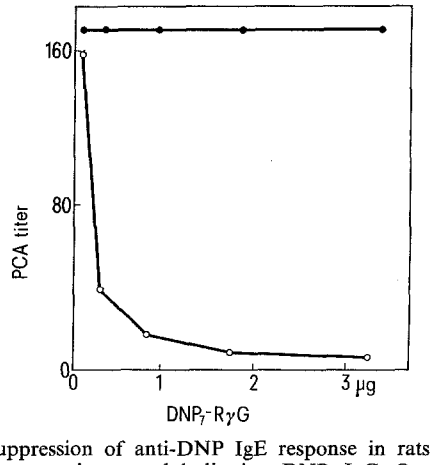
Summary. The induction of the anti-DNP IgE in rat was suppressed by pretreatment of rats with the tolerogen synthesized by coupling DNP to rat IgG, i.e.; DNP_{7-10}-IgG. It was found that DNP_{10}-IgG_1 was an effective tolerogen, whereas other DNP conjugates, i.e. DNP_9-IgM, DNP_9-IgA, DNP_{10}-IgE, DNP_{10}-IgG_{2c} and DNP_{10}-IgG_{2a} were ineffective.

It is generally believed that IgE as the main carrier of reaginic activity exerts its physiological function in vivo, resulting in the atopic disorders in man[3]. The immunosuppression of the production of IgE in vivo has been the subject of intensive investigation in immunotherapy. The study of the induction and function of reaginic antibody in animal systems is therefore considered as a major step towards understanding the fundamental biological role of human reaginic antibody. In numerous recent communications reported from our group we have shown that reaginic antibody responses to both hapten DNP and its carrier OA (ovalbumin) could be induced in mice, rats, guinea-pig and dogs[4-10] by a single i.p. injection of 1 µg of DNP_3-OA conjugate, and the formation of anti-DNP reaginic antibody could be successfully suppressed by treatment of mice or dogs with a conjugate of the hapten to isologous DNP-IgG. The characteristic feature of the immunosuppression of the IgE response of this system has been envisioned to have a great potential clinical significance in the treatment of allergic diseases in man[4,5]. In the present study, a similar system of the induction of reaginic antibodies in response

Table 1. Effect of different DNP_x-OA conjugates on the formation of IgE

Antigen (DNP-OA)*	PCA** titer DNP	OA
$DNP_{0.5}$-OA	0	0
$DNP_{2.8}$-OA	150	140
$DNP_{3.8}$-OA	160	140
DNP_{20}-OA	10	5

* Animals were immunized (o.p.) with 1 µg of DNP-OA in the presence of 1 mg $Al(OH)_3$ and 10^{10} *B. Pertussis.* ** Serum obtained from day 14 was used for measuring the IgE response by means of passive cutaneous anaphylaxis (PCA) assays in random outbred hooded rats. Animals were etherized for bleeding and for PCA assays.



Immunosuppression of anti-DNP IgE response in rats by DNP linked rat gamma immunoglobulin, i.e.; DNP_7-IgG. O——O, anti-DNP IgE response; ●——●, anti-OA IgE response. Serum obtained from day 14 was used for PCA assay.